

## Midazolam Injection

### DEFINITION

Midazolam Injection is a sterile solution of Midazolam Hydrochloride in Water for Injection or of Midazolam in Water for Injection prepared with the aid of Hydrochloric Acid. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of midazolam ( $C_{18}H_{13}ClFN_3$ ). It may contain Sodium Chloride, Benzyl Alcohol, and/or a chelating agent.

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

[NOTE—Protect all prepared Standard and sample solutions from light. ]

#### • PROCEDURE

**Buffer:** 6.7 g/L of dibasic sodium phosphate heptahydrate in water. Adjust with phosphoric acid to a pH of  $5.0 \pm 0.1$ .

**Solution A:** Prepare a filtered and degassed mixture of acetonitrile, methanol and *Buffer* (8:3:9).

**Solution B:** Acetonitrile and *Buffer* (3:1)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
20	0	100
35	0	100
37	100	0
45	100	0

**Standard solution:** Dissolve USP Midazolam RS in about 2 mL of methanol, and dilute quantitatively, and stepwise if necessary, with *Solution A* to obtain a 0.2-mg/mL solution.

**Sample solution:** [NOTE—The midazolam present in the Injection converts from the open-ring form to the closed-ring form when diluted with *Solution A*. The midazolam potency is determined based on the peak area of the closed-ring form. It takes approximately 60 min at  $40^\circ$  or 2–3 h at room temperature to complete the conversion. The *Standard solution* is not subject to this conversion process. ] Transfer a volume of Injection to a suitable volumetric flask, and dilute with *Solution A* to obtain a solution

containing about 0.2 mg/mL of midazolam . Transfer the resulting solution into suitable crimp top vials, seal tightly, and heat at about 40° for 60 min. Allow this solution to cool to room temperature before injection.

**Chromatographic system**

(See *Chromatography* ( 621 ) , *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1.0 mL/min

**Injection size:** 50 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 5500 theoretical plates

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of labeled amount of  $C_{18}H_{13}ClFN_3$  in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Midazolam in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**IMPURITIES****Organic Impurities**

[NOTE—Protect all prepared Standard and sample solutions from light. ]

**• PROCEDURE**

**Buffer, Solution A, Solution B, Mobile phase, Sample solution, and**

**Chromatographic system:** Proceed as directed in the Assay.

**Standard stock solution:** Use *Standard solution* in the Assay.

**Standard solution:** 0.5 µg/mL USP Midazolam RS in *Solution A* from *Standard stock*

*solution*

**Control solution:** 0.1 µg/mL USP Midazolam RS in *Solution A* from *Standard solution*

**System suitability**

**Samples:** *Standard solution* and *Control solution*

**Suitability requirements**

**Tailing factor:** NMT 2.5 for midazolam peak, *Standard solution*

**Column efficiency:** NLT 5500 theoretical plates, *Standard solution*

**Signal-to-noise ratio:** NLT 10, *Control solution*

**Relative standard deviation:** NMT 8.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- $r_U$  = peak response of the individual impurity from the *Sample solution*
- $r_S$  = peak response of midazolam from the *Standard solution*
- $C_S$  = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)
- $C_U$  = nominal concentration of Midazolam in the *Sample solution* (mg/mL)
- $F$  = relative response factor; 0.51 for the peak eluting at a relative retention between 0.79 and 0.97 with respect to midazolam ; 1.0 for all other peaks

**Acceptance criteria**

**Individual known impurity:** NMT 0.5%

**Individual unknown impurity:** NMT 0.1%

**Total impurities:** NMT 1.0%

[NOTE—Disregard all solvent- and excipient-related peaks.]

**SPECIFIC TESTS**

• **BENZYL ALCOHOL CONTENT** (if present)

**Buffer:** 3.4 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

**Mobile phase:** Acetonitrile and *Buffer* (7:13)

**System suitability solution:** 0.05 mg/mL of USP Midazolam RS and 0.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

**Standard solution:** 0.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

**Sample solution:** Transfer a measured volume of Injection to a suitable volumetric flask. Dilute with *Mobile phase* to obtain a concentration of about 0.5 mg/mL of benzyl alcohol, based on the labeled content of benzyl alcohol in the Injection.

**Chromatographic system**

(See *Chromatography* 〈 621 〉, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; L1 packing

**Flow rate:** 1.0 mL/min

**Injection size:** 50 µL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 6.0 between benzyl alcohol and midazolam

**Tailing factor:** NMT 2.0 for benzyl alcohol

**Relative standard deviation:** NMT 2.0% for benzyl alcohol

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzyl alcohol in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of benzyl alcohol from the *Sample solution*

$r_S$  = peak response of benzyl alcohol from the *Standard solution*


$C_S$  = concentration of USP Benzyl Alcohol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzyl alcohol in the *Sample solution* (mg/mL)

**Acceptance criteria:** The content of benzyl alcohol meets the requirements for *Added Substances* under *Injections* 〈 1 〉.

- **PARTICULATE MATTER IN INJECTIONS** 〈 788 〉: Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** 〈 85 〉: It contains NMT 8.33 USP Endotoxin Units/mg of midazolam.
- **PH** 〈 791 〉: 2.5–3.7
- **STERILITY TESTS** 〈 71 〉: Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Injections* 〈 1 〉.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type 1 glass.  
Store between 15° and 30°.
- **LABELING:** Label to indicate the vehicle used and the names and concentrations of any added preservatives. Indicate if the product is preservative free.
- **USP REFERENCE STANDARDS** { 11 }  
USP Benzyl Alcohol RS   
USP Endotoxin RS  
USP Midazolam RS

**Auxiliary Information**— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Mary S. Waddell Scientific Liaison 1-301-816-8124	(SM42010) Monographs - Small Molecules 4
{ 85 }	Radhakrishna S Tirumalai, Ph.D. Principal Scientific Liaison 1-301-816-8339	(GCM2010) General Chapters - Microbiology
{ 71 }	Radhakrishna S Tirumalai, Ph.D. Principal Scientific Liaison 1-301-816-8339	(GCM2010) General Chapters - Microbiology
Reference Standards	RS Technical Services 1-301-816-8129 rstech@usp.org	

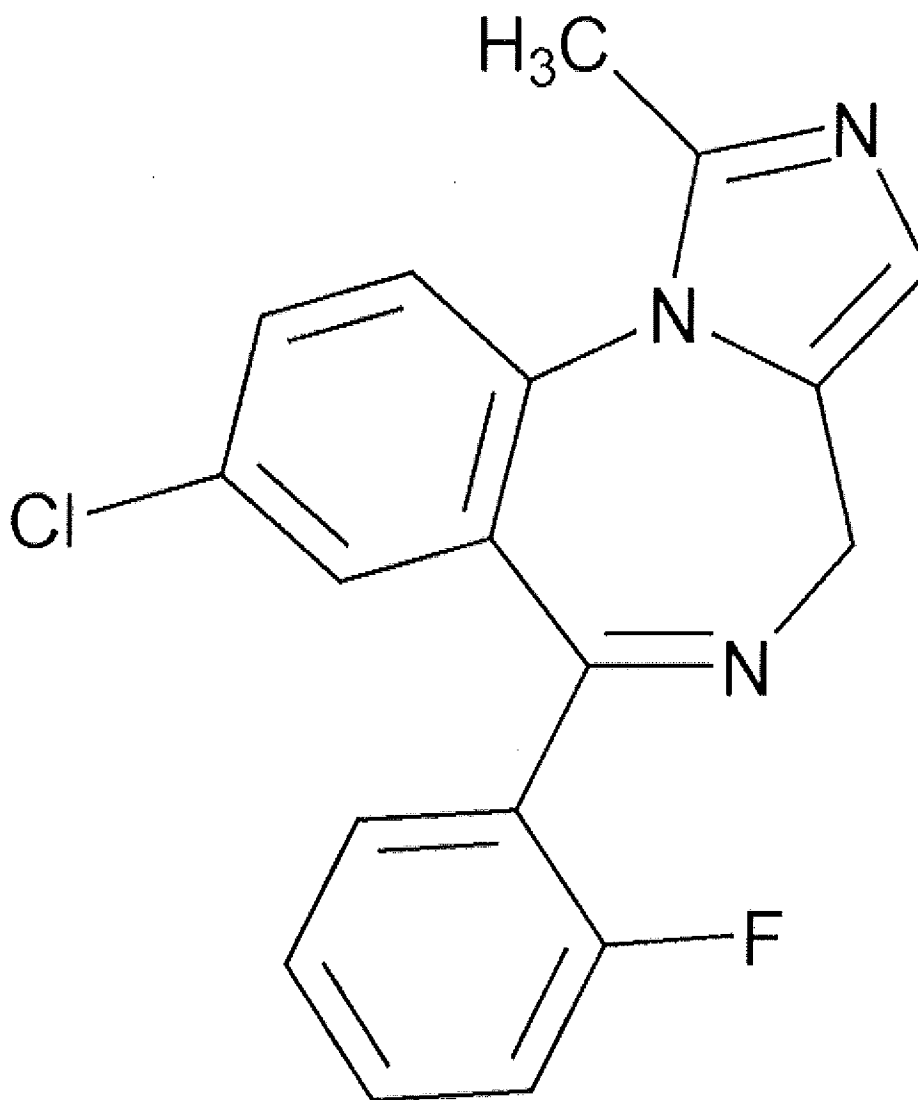
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**Chromatographic Column—**MIDAZOLAM INJECTION

Chromatographic columns text is not derived from, and not part of, USP 34 or NF 29.

## Midazolam



USP Image 3D Image

$C_{18}H_{13}ClFN_3$  325.77

4-*H*-Imidazo[1,5-*a*][1,4]benzodiazepine, 8-chloro-6-(2-fluorophenyl)-1-methyl;

8-Chloro-6-(*o*-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine [59467-70-8].

**DEFINITION**

Midazolam contains NLT 98.5% and NMT 101.5% of  $C_{18}H_{13}ClFN_3$ , calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION ( 197K )**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

## ASSAY

### • PROCEDURE

**Buffer:** 7.7 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of  $5.5 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (1:2)

**Standard solution:** 0.04 mg/mL of USP Midazolam RS in *Mobile phase*

**Sample solution:** 0.04 mg/mL of Midazolam in *Mobile phase*

### Chromatographic system

(See Chromatography ( 621 ) , System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L60

**Flow rate:** 1.5 mL/min

**Injection size:** 25 μL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Column efficiency:** NLT 10,000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{18}H_{13}ClFN_3$  in the portion of Midazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Midazolam in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.5%–101.5% on the dried basis

## IMPURITIES

**Inorganic Impurities**

- **RESIDUE ON IGNITION** ( 281 ): NMT 0.1%

**Organic Impurities**

- **PROCEDURE**

**Buffer, Mobile phase, Standard solution, and Chromatographic system:** Proceed as directed in the Assay.

**Sensitivity check solution:** Dilute the *Standard solution* with *Mobile phase* to obtain a 0.2-µg/mL solution.

**Sample solution:** 0.2 mg/mL of Midazolam in *Mobile phase*

**System suitability**

**Samples:** *Standard solution* and *Sensitivity check solution*

**Suitability requirements**

**Column efficiency:** NLT 10,000 theoretical plates, *Standard solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Peak ratio:** The ratio of the area of the midazolam peak of the *Standard solution* to the area of the midazolam peak of the *Sensitivity check solution* should be within 160–240.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Midazolam taken:

$$\text{Result} = (r_U/F)/[\Sigma(r_U/F) + r_T] \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_T$  = peak response of Midazolam from the *Sample solution*

$F$  = relative response factor (see *Impurity Table 1*)

**Acceptance criteria:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Reduced midazolam <sup>a</sup>	0.20	1.0	0.1
Reduced reduced midazolam <sup>b</sup>	0.24	1.0	0.1
Amino compound <sup>c</sup>	0.25	0.5	0.1
Oxide midazolam <sup>d</sup>	0.46	1.3	0.1

Nitromethylene compound <sup>e</sup>	0.76	1.0	0.1
Dihydropyridazinolam <sup>f</sup>	0.83	0.5	0.1
Midazolam	1.0	—	—
Desfluoromidazolam <sup>g</sup>	1.14	1.0	0.2
6 <i>H</i> -isomer <sup>h</sup>	2.48	0.7	0.1
Unknown impurity	—	1.0	0.1
Total impurities	—	—	0.5
<sup>a</sup> 8-Chloro-3a,4-dihydro-6-(2-fluorophenyl)-1-methyl-3 <i>H</i> -imidazo[1,5- <i>a</i> ][1,4]-benzodiazepine.			
<sup>b</sup> 8-Chloro-6-(2-fluorophenyl)-3a,4,5,6-tetrahydro-1-methyl-3 <i>H</i> -imidazo[1,5- <i>a</i> ][1,4]-benzodiazepine.			
<sup>c</sup> 2-Aminomethyl-7-chloro-2,3-dihydro-5-(2-fluorophenyl)-1 <i>H</i> -1,4-benzodiazepine.			
<sup>d</sup> 8-Chloro-6-(2-fluorophenyl)-1-methyl-4 <i>H</i> -imidazo[1,5- <i>a</i> ][1,4]-benzodiazepine-5-oxide.			
<sup>e</sup> 7-Chloro-1,3-dihydro-2-nitromethylene-5-(2-fluorophenyl)-2 <i>H</i> -1,4-benzodiazepine-4-oxide.			
<sup>f</sup> 8-Chloro-6-(2-fluorophenyl)-5,6-dihydro-1-methyl-4 <i>H</i> -imidazo[1,5- <i>a</i> ][1,4]-benzodiazepine.			
<sup>g</sup> 8-Chloro-6-phenyl-1-methyl-4 <i>H</i> -imidazo-[1,5- <i>a</i> ][1,4]-benzodiazepine.			
<sup>h</sup> 8-Chloro-6-(2-fluorophenyl)-1-methyl-6 <i>H</i> -imidazo[1,5- <i>a</i> ][1,4]-benzodiazepine.			

## SPECIFIC TESTS

- **LOSS ON DRYING** ( 731 ): Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ( 11 )  
USP Midazolam RS

**Auxiliary Information**— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Mary S. Waddell Scientific Liaison 1-301-816-8124	(SM42010) Monographs - Small Molecules 4
Reference Standards	RS Technical Services 1-301-816-8129 <a href="mailto:rstech@usp.org">rstech@usp.org</a>	

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**Chromatographic Column—**

MIDAZOLAM

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